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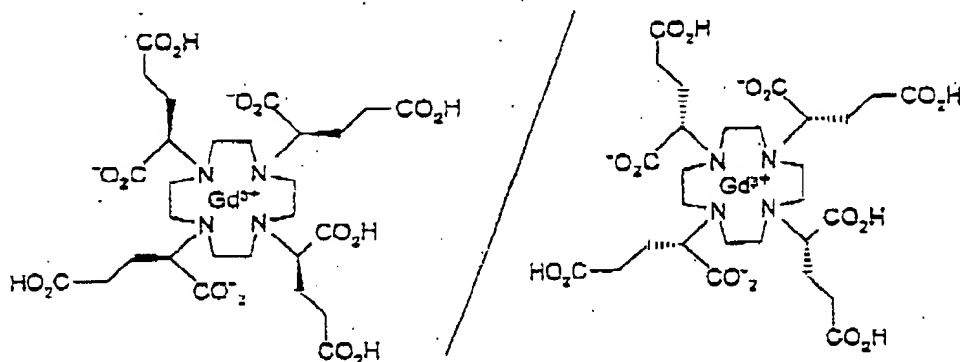
WO 00/71526

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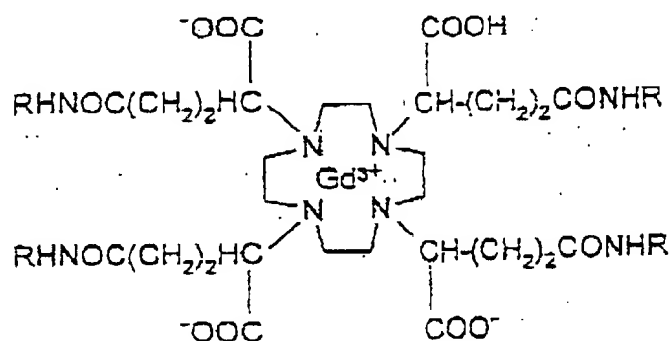
TETRAMIDES OF A GADOLINIUM COMPLEX AND APPLICATION IN  
MEDICAL IMAGING

*Insai*

The present invention relates to tetramides which are  
5 derived from the pair of RRRR/SSSS enantiomers of  
tetra( $\alpha$ -carboxyethyl)gadoterate, represented by the  
formulae

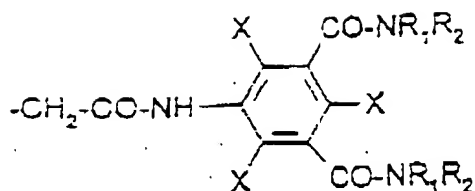


10 It is disclosed in EP 0 661 279 that the amides of  
formula II

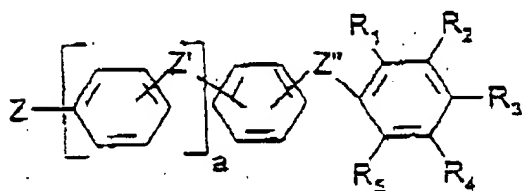


in which R is a bulky hydrophilic group with a  
molecular mass of greater than 200, exhibit a  
15 longitudinal relaxivity  $r_1$  which is markedly superior  
to those of the chelates not carrying the bulky side  
group  $(CH_2)_2CONHR$  and can be used as contrast agents in  
magnetic resonance diagnostic imaging.

20 WO 97/01359 relates to the products of formula II in  
which R is a group of formula



Application EP 98 403108 of 9 December 1998 relates to the products of formula II in which R is the following group



It is known that the relaxivity  $r_1$  of a gadolinium chelate is a complex function of various more or less independent factors, including the electronic correlation time, rotation correlation time and water exchange time, which factors depend in particular on the spatial structure of the chelating agent around the paramagnetic cation, so that 2 stereoisomers can have substantially different relaxivities.

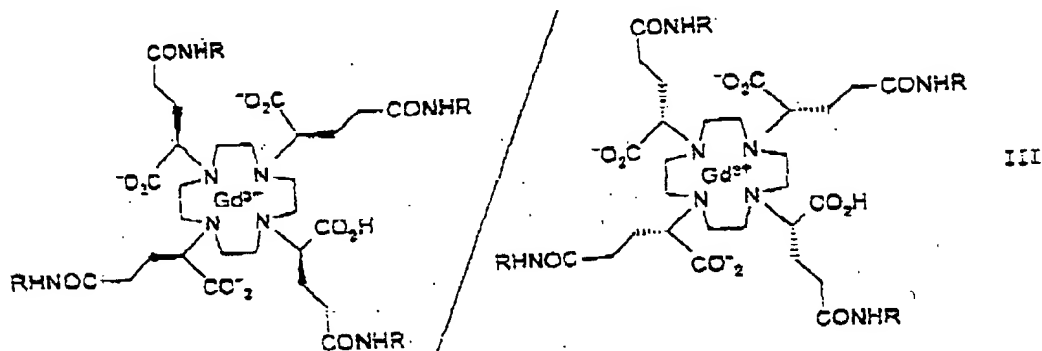
Furthermore, it is essential for the specific characteristics of a pharmaceutical product to be reproducible in terms of effectiveness and of toxicity between successive manufacturing batches and it can be difficult to ensure such reproducibility in the presence of numerous stereoisomers because of their substantial differences in chemical reactivity and in physical properties.

It was thus desirable to find a process which makes it possible, at the industrial stage under acceptable economic conditions, to obtain a mixture of stereoisomers of the amides of formula II in exactly defined proportions and thus to isolate, with good yields, one of the possible racemic compounds which

does not comprise the other stereoisomers and which exhibits an advantageous relaxivity  $r_1$  in the range of the fields currently used clinically, namely between 0.5 and 1.5 tesla.

5

The racemic compounds according to the invention are represented by the formula III



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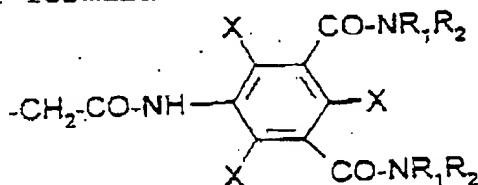
in which R is a phenyl group or (C<sub>1</sub>-C<sub>8</sub>) alkyl group which are substituted or interrupted by one or more groups selected from phenyl, alkyl, oxy, amino or amido groups, which may or may not be substituted by alkyl,

15 it being possible for the phenyl groups to be substituted by OH, Br, Cl, I, (C<sub>1</sub>-C<sub>8</sub>)alkyl, (C<sub>1</sub>-C<sub>8</sub>)alkyleneoxy, NO<sub>2</sub>, NR<sub>x</sub>R<sub>y</sub>, NR<sub>x</sub>COR<sub>y</sub>, CONR<sub>x</sub>R<sub>y</sub> or COOR<sub>x</sub>, R<sub>x</sub> and R<sub>y</sub> being (C<sub>1</sub>-C<sub>8</sub>)alkyl or H,

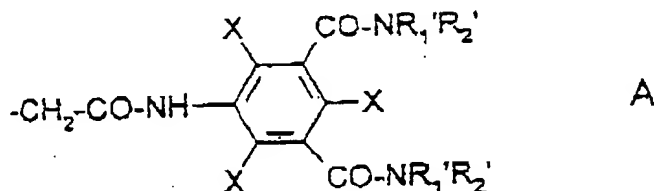
20 and it being possible for the linear or branched or cyclic alkyl groups to be hydroxylated, and the salts of these acids with inorganic or organic bases, such as NaOH, KOH, N-methylglucamine, tris-(hydroxymethyl)aminomethane, lysine or diethanolamine.

25 Preference is given, among these, to the compounds in which

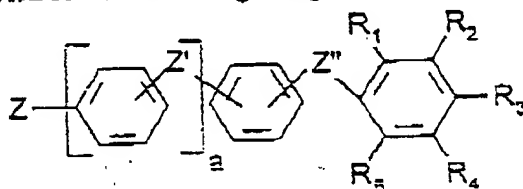
R is a group of formula



in which X is Br or I, R<sub>1</sub> is H, (C<sub>1</sub>-C<sub>3</sub>)alkyl or (C<sub>2</sub>-C<sub>8</sub>)mono- or polyhydroxyalkyl and R<sub>2</sub> is (C<sub>2</sub>-C<sub>8</sub>)mono- or polyhydroxyalkyl, or else R<sub>1</sub> is H and R<sub>2</sub> is a group of formula



- 5 X being as defined above and R'<sub>1</sub> and R'<sub>2</sub> taking any one of the meanings given for R<sub>1</sub> and R<sub>2</sub>, with the exception of A, it being understood that -CO-NR<sub>1</sub>R<sub>2</sub> or -CO-NR'<sub>1</sub>R'<sub>2</sub> comprise at least two hydroxyl groups,  
10 and those in which R is a group



- in which a is 1 or 2,  
Z is a bond, CH<sub>2</sub>, CH<sub>2</sub>CONH or (CH<sub>2</sub>)<sub>2</sub>NHCO,  
Z' is a bond, O, S, NQ, CH<sub>2</sub>, CO, CO-NQ, NQ-CO, NQ-CO-NQ  
15 or CO-NQ-CH<sub>2</sub>-CONQ,  
Z'' is CO-NQ, NQ-CO, CO-NQ-CH<sub>2</sub>-CO-NQ or NQ-CO-CH<sub>2</sub>-NQ-CO,  
with Q being H or an optionally hydroxylated (C<sub>1</sub>-C<sub>4</sub>)alkyl group,  
R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub>, independently of one another, are  
20 selected from H, Br, Cl, I, CO-NQ<sub>1</sub>Q<sub>2</sub> or N(Q<sub>1</sub>)-CO-Q<sub>2</sub>, and  
Q<sub>1</sub> and Q<sub>2</sub>, which are identical or different, are selected from optionally hydroxylated (C<sub>2</sub>-C<sub>6</sub>)alkyl groups optionally interrupted by an oxygen atom, so that Q<sub>1</sub> and Q<sub>2</sub> together comprise from 4 to 10 OH groups,  
25 it being understood that at least 1 and at most 2 R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> groups are amide groups.

The racemic compounds of the invention can be prepared by a method known per se by reaction of the amine RNH<sub>2</sub>  
30 with the pair of complexes of the enantiomeric octaacids of formula I in aqueous solution with an

agent which activates carboxyl functional groups under conventional conditions for peptide condensations, as disclosed in the abovementioned patents, for mixtures of isomers.

5  
Some of the isomers of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid), obtained by hydrolysis of the corresponding ethyl esters, separated by silica liquid chromatography and crystallization from water,  
10 have been described by Judith A.K. Howard et al. in Chem. Commun., 1381-1382 (1998).

A process which can be operated industrially has now been found which makes it possible to obtain the pair  
15 of RRRR/SSSS enantiomers starting from the mixture of the stereoisomers of the gadolinium complex of this octaacid resulting from the substitution, by a conventional method, of the nitrogen atoms of 1,4,7,10-tetraazacyclododecane. It consists in carrying out the  
20 isomerization by simple heating in aqueous solution at acidic pH, preferably between 2 and 4.5 and better still between 2.5 and 3.5, and at a temperature of greater than 70°C, preferably of greater than 90°C and better still at reflux of the solution, for the time  
25 needed to obtain the racemic compound of the invention, i.e. from a few hours to a few days, in particular 35 to 45 hours at reflux at approximately pH 3.

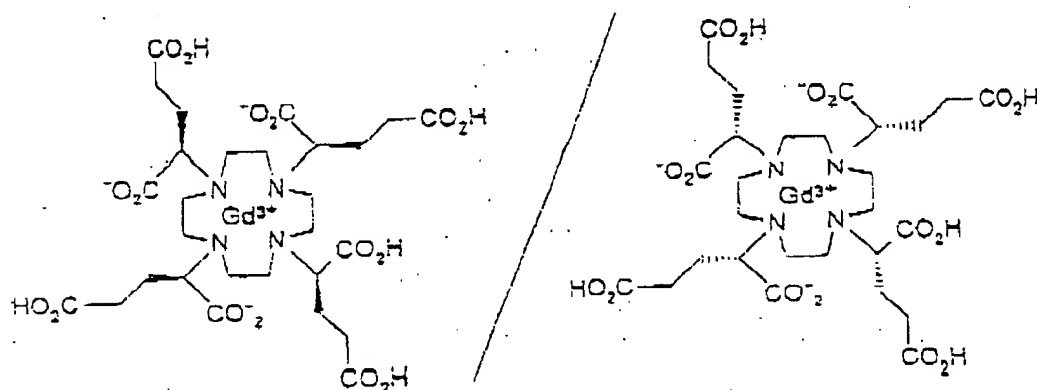
The starting mixture of the stereoisomers can be  
30 obtained by the action of the compound of formula  $R'OOC-CHX-(CH_2)_2-COOR'$ , in which  $R' = H$  or  $(C_1-C_3)$ alkyl and X is a leaving group in a nucleophilic substitution, in particular a halogen atom, preferably bromine, or a sulphonate or tosylate or triflate group,  
35 which reaction is followed by the hydrolysis of the ester functional groups, in particular by the action of an alkaline carbonate or hydroxide in an alcoholic, aqueous/alcoholic or aqueous medium.

A person skilled in the art will select, during preliminary trials, the concentration of the solution, the pH, the temperature and the duration of the heating in order to carry out complete isomerization without significant decomposition, in particular according to the product and the amount treated.

It is surprising that, under these conditions, the chelate is not decomplexed and that the decomposition of the ligand is negligible and that, in addition, the pair of enantiomers which is finally isolated comprise less than 15% of the 3 pairs formed on conclusion of a conventional synthesis, which consists in hydrolysing, in basic medium, the product obtained by reaction of ethyl 2-bromoglutarate with the heterocycle and in then carrying out its complexation by the action of  $GdCl_3$  or  $Gd_2O_3$ .

Thus, according to another of its aspects, the present invention relates to a process comprising the stages consisting:

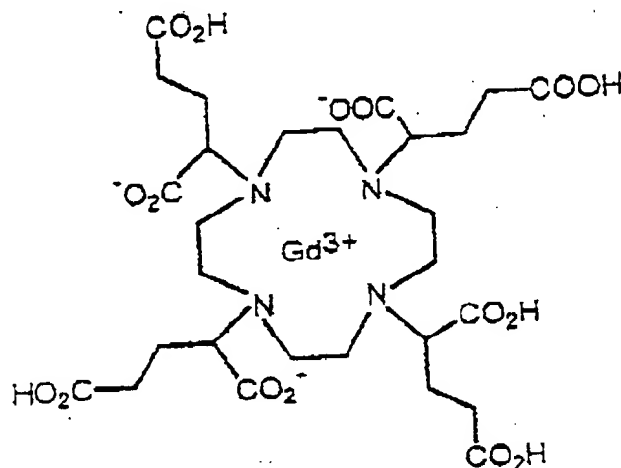
1 - in keeping an aqueous solution of the mixture of the stereoisomers of the gadolinium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid), with a pH of between 2 and 4.5, at a temperature of greater than  $70^\circ C$  for a few hours to a few days, so as to obtain the racemic mixture of octaacids of formula:



2 - in reacting this mixture with the amine  $\text{RNH}_2$ , R being defined above for the formula III, with an agent which activates the acid functional group.

5

The starting mixture of the stereoisomers of the gadolinium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid) of formula:



10 can be obtained in a simple fashion by employing a process comprising the stages consisting in:

- reacting 1,4,7,10-tetraazacyclododecane with a compound of formula  $\text{R}'\text{OOC}-\text{CHX}(\text{CH}_2)_2-\text{COOR}'$  in which  $\text{R}'$  is a hydrogen atom or  $(\text{C}_1-\text{C}_3)\text{alkyl}$  and X is a leaving group;

15 - conventionally hydrolysing the ester functional group of the resulting compound when  $\text{R}'$  is other than H; and  
- complexing the compound thus obtained with the gadolinium ion.

20

Mention may be made, as leaving group which can be used, of the sulphonate, tosylate and triflate groups.

25 The invention also relates to compositions for nuclear magnetic resonance medical imaging which comprise the racemic compounds of the invention in combination with conventional vehicles and additives. The doses at which these contrast agents will be administered depend on

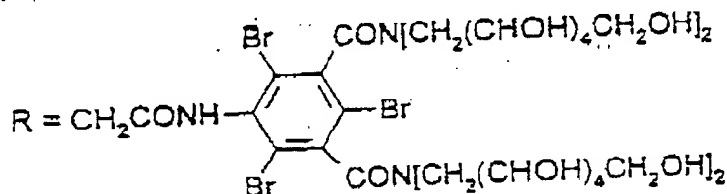
their magnetic efficiency, on their biodistribution and on their administration route, as on the size of the subject, on the organ to be observed and on the nature of the pathology. For an intravascular administration, the unit concentration will be between 0.5 and 5 mM for an adult, presented in aqueous solution.

In that which follows, examples of the preparation of the compounds of the invention are described.

The isolated products are characterized by their retention times ( $t_r$ ) in high performance liquid chromatography (HPLC). Their molecular masses were determined by mass spectrometry (electrospray).

#### Example 1

Compound of formula II in which



A. Gadolinium chelate of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(2-glutaric acid) (mixture of the 6 diastereoisomers).

1. - 30 g of sodium carbonate and then 78 g of ethyl 2-bromoglutarate, prepared, for example, as described in Acta Chim. Acad. Sci. Hung., 41(3), 331-6 (1964), are introduced into a solution of 25 g of 1,4,7,10-tetraazacyclododecane in 280 ml of acetonitrile. The medium is brought to its reflux temperature for one day, during which 78 g of the brominated derivative with 30 g of sodium carbonate are added on two occasions. After cooling, the precipitate is filtered off and the organic phase is washed with water before being extracted with a dilute aqueous hydrochloric acid solution. The aqueous phase, brought to approximately



pH 3-4, is subsequently extracted with toluene.

The desired product is purified by silica chromatography, elution being carried out with  
5 methylene chloride, optionally as a mixture with acetone.

## 2. - Hydrolysis of the ester functional groups:

10 46 g of the octaester are introduced into a solution of 52 ml of ethanol and 350 ml of water, to which 50 g of NaOH pearls have been added.

15 After stirring for two days at 80°C, 500 ml of cation exchange resin in the weak acid form are introduced into the cooled solution for neutralization and then, after separation of the solid phase, 500 ml of anionic exchange resin in the strong base form are introduced. The resin is separated and introduced into 500 ml of 6N  
20 aqueous acetic acid solution; the final product, which has passed into solution, is isolated in the form of a powder by evaporating the solvent under vacuum.  
HPLC: 25 cm x 4.6 mm column of Nucleosil® C18 - 100-5 µm silica gel.

25 Eluent: aqueous H<sub>2</sub>SO<sub>4</sub> (0.1%) for 10 minutes and then with 0 to 10% (v/v) of CH<sub>3</sub>CN over 10 minutes: flow rate = 1 ml/min; T = 25°C;

$t_r$  = 5.4, 8.7, 10.2 and 14 minutes (isomers)

(CH<sub>3</sub>COOH -  $t_r$  = 4.5 minutes).

30

## 3. - Complexation:

35 With gadolinium oxide: 0.47 g of gadolinium oxide is introduced into 30 ml of a solution, at a pH of 5.5 to 6, of 2 g of the preceding octaacid and the mixture is maintained at 80°C for 3 hours, during which the pH is adjusted, if necessary. The medium is filtered, concentrated to a third and then poured into 100 ml of ethanol. The precipitate formed can be purified by

treatment with a weak basic resin before another precipitation from ethanol.

With gadolinium chloride: the mixture of 6.5 g of the  
5 octaacid and 3.5 g of  $GdCl_3 \cdot 6H_2O$  in 130 ml of water is  
brought to pH 6.5 by addition of aqueous NaOH (1N) and  
is brought to 60°C for 2 hours, during which the pH is  
maintained at 6.5 by addition of a total of 21 ml of 1N  
aqueous NaOH. After a few hours at ambient temperature,  
10 the mixture is concentrated to 25 ml and the final  
product is precipitated from 250 ml of  $C_2H_5OH$  before  
being purified.

HPLC: 25 cm x 4 mm Symmetry® - RP 18 - 5  $\mu$ m column  
(Waters®)

15 UV detector at 200 nm

mobile phase: 0.037N aqueous  $H_2SO_4$  with  $CH_3CN$  gradient  
(from 0% to 20% over 60 minutes); flow rate 1 ml/minute

pair of isomers (a) (30%)\*  $t_r$  = 28-32 minutes

pair of isomers (b) (65%)\*  $t_r$  = 32-36 minutes

20 pair of isomers (c) (5%)\*  $t_r$  = 37-41 minutes

\* percentage in the mixture, expressed with respect to  
the areas under the curve.

### B. Isomerization of the preceding mixture:

25

A solution of 10 g of the preceding mixture in 100 ml  
of refluxing water is acidified by addition of HCl (1N)  
to pH 3. After stirring for 42 hours at this  
temperature, the solution is concentrated under reduced  
30 pressure to a volume of 10 ml and left to return to  
ambient temperature. 6 g of precipitated final product  
are isolated by filtration, which product comprises a  
trace of isomers (b). It can be purified by  
recrystallization from water.

35

If heating is carried out at only 80°C, 30% of the pair  
(b) still remains after heating for 150 hours and 10%  
still remains after 400 hours.

C. Amidation:

0.46 g of the pair of isomers obtained above and 2 g of  
 N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-  
 5 (glycylamino)isophthalamide (compound IIId of  
 WO 97/01359) are dissolved in 8 ml of water, and a 6N  
 aqueous NaOH solution is poured into the medium to pH 6  
 before introducing, at 40°C, 0.48 g of 1-(3-  
 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.  
 10 The medium is maintained at 40°C for 2 hours with  
 stirring while introducing, from time to time, an N  
 aqueous NaCl solution in order for the pH not to exceed  
 7.

15 After returning to ambient temperature, the solution is  
 poured into 100 ml of ethanol and the precipitate  
 formed is isolated and then dissolved in 100 ml of  
 water to produce a solution. This solution is subjected  
 to tangential ultrafiltration through a polyether-  
 20 sulphone membrane, the cutoff threshold of which is 1  
 Kdalton, in a Minisette® cell sold by Filtron®, USA.

After lyophilization, 1.5 g of the final product are  
 isolated in the form of a white powder.

25 HPLC: 25 cm x 4 mm Symmetry® - RP 18 - 5 µm column  
 (Waters®)

UV detector at 230 nm

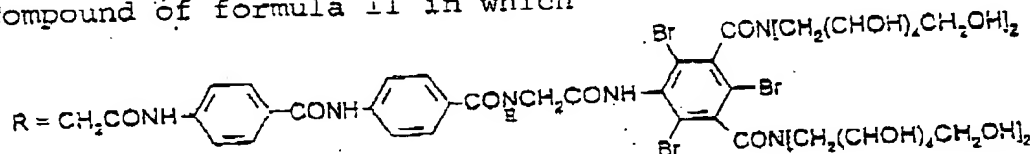
mobile phase: 0.037N aqueous H<sub>2</sub>SO<sub>4</sub> with CH<sub>3</sub>CN, gradient  
 from 99/1 to 90/10 (v/v) over 25 minutes, flow rate

30 1 ml/minute,

t<sub>r</sub> = 16 to 20 minutes (several peaks).

Example 2

35 Compound of formula II in which



A. N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tri-bromo-5-(4-[4-(aminoacetamido)benzamido]benzoylglycyl-amino)isophthalamide.

5 (a) 4-[4-Nitrobenzamido]benzoic acid:

100 g of 4-nitrobenzoyl chloride are introduced, little by little, into 74 g of 4-aminobenzoic acid and 360 ml of dimethylacetamide while maintaining the temperature at less than 25°C. After stirring for 24 hours, 500 ml of methylene chloride are added at 10°C to precipitate the desired product. After washing with water and drying, 145 g of product are isolated.

15 (b) 4-[4-Aminobenzamido]benzoic acid;

A suspension of 136 g of the preceding acid in 1.8 litres of water, to which 240 ml of 1N aqueous NaOH solution and 14 g of palladium-on-charcoal (10%) have been added, is subjected to a hydrogen pressure of 0.6 MPa for 4 hours. The pH of the final suspension is then brought to approximately 10 before filtering through Celite® to remove the catalyst. The precipitate formed during the acidification of the filtrate to pH 5.3 is isolated and dried.

w = 106 g; M.p. > 260°C.

(c) 4-[4-(Phthalimidoacetamido)benzamido]benzoic acid:

32 ml of thionyl chloride are introduced dropwise into a solution of 90 g of phthalimidoacetic acid in 400 ml of dimethylacetamide at 10°C and then, after stirring for 3 hours, 105 g of the amino acid obtained above are introduced at a temperature of less than 20°C. After stirring for 12 hours, the medium is poured into 4 litres of water and the isolated precipitate is washed with hot water.

Weight after drying: 176 g; M.p. > 260°C.

(d) Chloride of the preceding acid:

2.5 ml of thionyl chloride are introduced into 10 g of the acid, in suspension in 50 ml of dioxane and 1 ml of dimethylformamide, and the mixture is kept stirred at 50°C for 5 hours. After addition of one volume of diisopropyl ether, 10 g of precipitate are isolated.

The acid can also be suspended in toluene with tricaprylylmethylammonium chloride as catalyst.

(e) N,N'-Bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-(4-[4-(phthalimidoacetamido)benzamido]-benzoylglycylamino)isophthalamide:

A solution of 2.25 g of acid chloride with 5 g of N,N'-bis(2,3,4,5,6-pentahydroxyhexyl)-2,4,6-tribromo-5-(glycylamino)isophthalamide and 0.7 ml of triethylamine in 25 ml of N-methylpyrrolidone is kept stirred for 12 hours; the  $(C_2H_5)_3N \cdot HCl$  precipitate is then separated by filtration.

(f) Hydrazinolysis:

A solution of 1.4 equivalents of hydrazine hydrate in 6 ml of water is introduced into the preceding phthalimide solution at 70°C. After stirring for 2 hours at 90°C, the cooled mixture is poured into 125 ml of ethanol. 9 g of precipitate are isolated, from which the phthalylhydrazide is separated by precipitation of an aqueous solution at pH 2, before ultrafiltration at pH 6 through a polyamide membrane to remove the impurities of low mass. The final hydrochloride is subsequently isolated by lyophilization.

Yield: 50% from the acid chloride.

HPLC: 25 cm x 4 mm Lichrospher® 100 Å - C18 - 5 µm column (Merck, Germany).

Eluent:  $CH_3COOH$  in  $H_2O$  (pH 3.3) and  $CH_3CN$  (90/10 v/v); flow rate 1 ml/min;

$t_r$  = 22, 24 and 27 minutes (3 peaks).

5 B. 0.28 g of the complex obtained in stage (B) of the preceding example and 2 g of the hydrochloride obtained in the preceding stage (A) are dissolved in 12.4 ml of water and the pH of the solution is brought to 6 by addition of N aqueous NaOH before adding 10 ml of a solution in dioxane of 0.2 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.024 g of hydroxybenzotriazole.

10 The solution is then stirred for 4 hours at ambient temperature while maintaining its pH at approximately 6, before being poured into 100 ml of ethanol. The precipitate formed is dissolved in 100 ml of water and the solution is ultrafiltered through a polyethersulphone membrane with a cutoff threshold of 30 Kdaltons.

20 After removing the solvent, 1.3 g of the desired product are obtained in the form of a white powder.  
HPLC: 25 cm x 6 mm Zorbax® - 300 5B - C18 - 5  $\mu$ m column (Hewlett-Packard)  
UV detector: 290 nm  
25 Eluent: aqueous  $\text{CH}_3\text{COONH}_4$  (0.005M) with a  $\text{CH}_3\text{CN}$  gradient (90/10 to 82/18) (v/v) over 60 minutes; flow rate 1 ml/min;

$t_r$  = 30 to 40 minutes (several peaks).